malignancies (see 9.2 of Exhibit C, Stuart J. Schnitt, "Pathology of Breast Cancer", Atlas of Breast Cancer 9.2,(2000), pp. 9.2 – 9.11).

Further discussed in Exhibit B is that the GERP gene maps to chromosome $10_q24.3$, a region showing frequent deletion or loss of heterogygosity in glioblastomas. This locus is thought to harbor tumor suppressor genes, indicating that this novel molecule may be a new tumor suppressor gene important in gliomas and other malignancies, including breast cancer. Thus, it is clear that BS203 has utility as a diagnostic tool in detecting breast cancer. In light of the above amendments and remarks, Applicant respectfully submits that the new claims are in a condition for allowance and requests that this rejection be withdrawn.

Claims 9, 13-20 and 24 are rejected under 35 U.S.C. 112, first paragraph as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. The Examiner's rejection is based on 90% identity language. In an effort to expedite prosecution, claims 9, 13-20 and 24 have been cancelled and new claims 25-34 do not include "percent identity" language. Furthermore, new claims 25-32 encompass language which refers to equivalent degenerate coding sequences thereof. The degeneracy of the genetic code is a concept that is well known to those skilled in the art and is even discussed in section 2144.09 of the February 2000 revision of the Manual for Patent Examining Procedure as "the fact that most amino acids are specified by more than one nucleotide sequence or codon." Applicant respectfully submits that the new claims are in a condition for allowance and requests that this rejection be withdrawn.

The Examiner further states that "the specification does not identify any epitopes and no guidance is provided as to how one of skill in the art would select appropriate fragments of a protein which function as an epitope". Applicant seeks to clarify that

methods for identifying epitopes in a novel peptide sequence are well known and described in both the scientific, commercial, and patent literature. For example, M. H. Van Regenmortel describes how to predict epitopes from the primary sequence of a protein. (See "Protein structure and antigenicity", *Int J Rad Appl Instrum B.*, 14(4):277-80, 1987.)

Further, Perkin-Elmer Biosystems, a major provider of DNA sequencing and peptide synthesizing instruments has established a public website which describes how to select peptides which reflect the epitopes of a protein. (See http://www.pebio.com/pa/340913/html/chapt2.html#Choosing the Epitope.) This electronic publication was posted in 1996 and basically describes the process employed by the inventors of the current patent application.

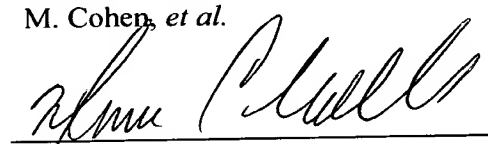
In addition, patent application PCT/US97/00485 describes in detail how to identify epitopes from peptide sequences. The sequence can be scanned for hydrophobicity and hydrophilicity values by the method of Hopp, Prog. Clin. Biol. Res. 172B: 367-377 (1985) or the method of Cease et al, J. Exp. Med. 164: 1779-1784 (1986) or the method of Spouge et al, J. Immunol. 138: 204-212 (1987). Commercial software programs to implement these methods are available. One skilled in the art would clearly understand how to determine antigenic regions in a novel polypeptide, including the claimed BS203.

The Examiner states that the amendments to the claims limiting to a "breast tissue-specific polynucleotide comprising "anyone of SEQUENCE ID NOS: 1-14" raises new issues under 35 U.S.C. 112, first paragraph. This language has been deleted from the claims.

CONCLUSION

In view of the aforementioned amendments and remarks, Applicant respectfully submits that the above-referenced application is now in a condition for allowance and Applicant respectfully requests that the Examiner withdraw all outstanding objections and rejections and passes the application to allowance.

Respectfully submitted,
M. Cohen, *et al.*



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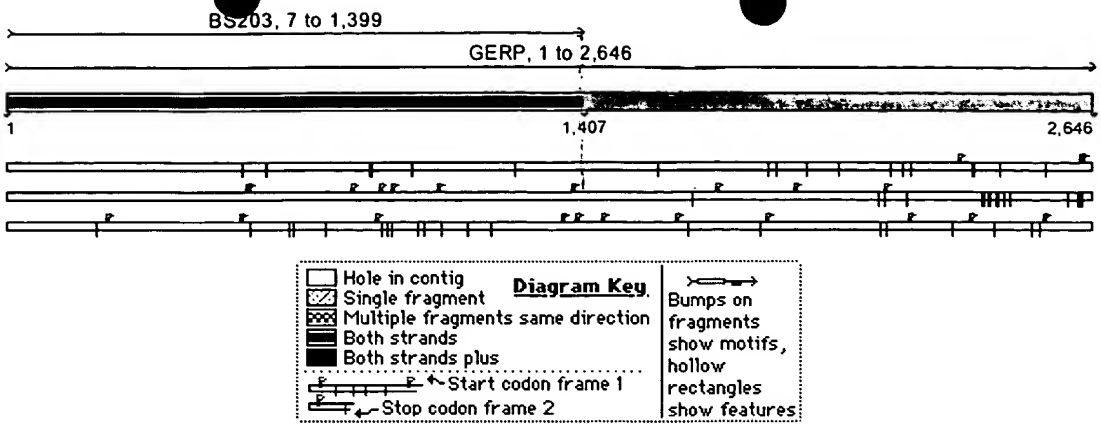

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EXHIBIT A

Biochemical and Biophysical Research Communications 279, 482–486 (2000)
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A Novel RING Finger-B Box-Coiled-Coil Protein, GERP

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Received November 13, 2000

The RING finger domain occurs in a wide variety of proteins involved in cellular regulation. The polymerase chain reaction was used to search for novel RING finger proteins, using primers derived from expressed sequence tags (ests). A cDNA encoding a novel RING finger protein expressed in brain, lung, breast, placenta, kidney, muscle, and germinal center B cells is described. The human gene is expressed in a variety of tumors, including anaplastic oligodendroglioma and maps to chromosome 10q24.3, a region showing frequent deletion or loss of heterozygosity in glioblastomas. It was therefore designated glioblastoma expressed RING finger protein (GERP). GERP contains an N-terminal RING finger, followed by two B-boxes and a coiled-coil, and thus belongs to the RBCC subfamily of RING finger proteins. The structure of this protein and its mapping to a locus thought to harbor tumor suppressor genes indicates that it may be a new tumor suppressor gene important in gliomas and other malignancies. © 2000 Academic Press

Key Words: **RING finger; RBCC; glioblastoma; 10q24.3.**

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The RING finger motif is a distinct zinc binding domain found in a wide variety of proteins. RING finger proteins have been shown to play important roles in signal transduction, transcriptional regulation, ubiquination and apoptosis (1, 2). An important subgroup of RING finger proteins is the RBCC group (3). These contain an N-terminal RING finger followed by one or two B-box domains, a second form of zinc finger. The B-box is then followed by a coiled-coil domain and a variable C-terminal region. RBCC proteins containing a single B-box include RFP (4), TERF (5), SS-A/RO (6), rpt-1 (7), AFP (8), BERP (9), and HERF1 (10).

```
AI830869    MAENWKNCFEEELICPICLHVFVEPVQLPCKHNFCRGCIGEAWAKDSGLVRCPECNQAYNQKPGLEKN
            LKLTNIVEKFNAXDVGKPPAALHCVFCRRGPPLPAQKVCLRCEAPLLQSHVQTHLQQPSTARGHLLVE
            ADDVRAWSCPQHNAYRLYHCEAEQVAVCQYCCYYSGAHXGHSVCDVEIR

AA523517    ADDVRAWSCPQHNQYRLYHCEAEQVAVCQYCCYYSGARQGHSVCDVEIRRNEIRKMLMKQQDRLEERD
            MDIEDQLYKLESDKRLVEEKVNQLKEEVRLQYEKLHQLLDEDLRQTVEVLDKAQAKFCSENAAQA

AW968881    -DIEDQLYKLESDKRLVEEKVNQLKEEVRLQYEKLHQLLDEDLRQTVEVLDKAQAKFCSENAAQALHL
            GERMQEAKKLLGSLQLLFDKTEDVSFMKNTKSVKILMDRTQTCTSSSLSPTKIGHLNSKLFLNEVAKK
            EKQLRKMLEGPFSTPVPFLQSVPLYPCGVSSSGAEKRKHSTAFPEASFLETSVGP

AW574584    EKQLRKMLEGPFSTPVPFLQSVPLYPCGVSSSGAEKRKHSTAFPEASFLETSSGPVGGQYGAAGTASG
            EGQSGQPLGPCSSTQHLVALPGGAQPVHSSPVFPPSQYPNGSAAQQPMLPQYGG

AW968886    ----------SSTQHLVALPGGAQPVHSSPVFPPSQYPNGSAAQQPMLP

AC006106    ----------GGAQPVHSSPVFPPSQYPNGSAAQQPMLPQYGGRKILVCSVDNCYCS
            SVANHGGHQPYPRSGHFPWTVPSQEYSHPLPPTPSVPQSLPSLAVRDWLDASQQPGHQD
            FYRVYGQPSTKHYVTS*

AA513682    SVANHGGHQPYPRSGHFPWTVPSQEYSHPLPPTPSVPQSLPSLAVRDWLDASQQPGHQD
            FYRVYGQPSTKHYVTS*
```

FIG. 1. Alignments of the predicted translation products for expressed sequence tags (ests) showing the assembly of these sequences into a predicted full-length human protein. The conserved N-terminal RING finger is in bold.

0006-291X/00 $35.00

A number of RBCC proteins have been described which possess a pair of B-box domains. PML was first identified as part of the translocation underlying promyelocytic leukemia (11–13). Similarly, the first of the TIF1 family of RBCC proteins, TIF1α, is transforming when fused with the B-RAF proto-oncogene (14, 15). These studies have suggested an important role for the RBCC domain in cellular transformation. Other proteins in this paired B-box family include the estrogen responsive finger protein (EFP) (16) and the Opitz syndrome MID1 (17) and related MID2 (18) proteins. The functions of these various proteins are still being determined, but one member of this group, ARD1, appears to be a novel GTPase (19).

We are using the polymerase chain reaction (PCR) method to identify novel RING finger proteins. Here we describe a novel RBCC protein which is highly conserved between mouse and human, and contains two B-box domains. This gene is expressed in a number of tumors, including gliomas, and maps to a region associated with glioblastomas, and has therefore been designated GERP (glioblastoma expressed RING finger protein).

MATERIALS AND METHODS

Primer design and PCR. We have searched the GenBank expressed sequence tag (est) databases for novel RING finger sequences. A novel RING finger sequence was obtained, and further screening of the database yielded 3′ and 5′ extensions to this sequence which could be assembled to yield a hypothetical full-length cDNA for mouse and human (Fig. 1). A forward primer was designed to include the first ATG downstream of a stop codon (5′-CATGGCGGAGAATTGGAAGA-3′), while the reverse primer lay downstream of the predicted stop codon of the human cDNA (5′-GGGTGTGAAGGGGAAGGAGT-3′). Reverse transcriptase-PCR analysis was first performed on human brain mRNA. PCR products were cloned into pCR2.1 (Invitrogen) and sequenced by dideoxy nucleotide chain termination.

Northern blot analysis. A human multiple tissue Northern blot containing about 1 μg of poly(A)$^+$ RNA per lane was used (Clontech). The membrane was hybridized with a ^{32}P-labeled probe derived from the full-length GERP cDNA using the Random Primers DNA Labeling System (Life Technologies). Northern blotting was carried out with the Northern Max Northern Blotting Kit (Ambion) and the autoradiograph exposed at −80°C with an intensifying screen.

RESULTS AND DISCUSSION

The initial reverse-transcriptase-PCR analysis was performed on human brain mRNA. As predicted, PCR using the primers designed from the ests, yielded a cDNA sequence of 1.9 kb. The initial methionine is within a strong consensus sequence for translation initiation (20). Translation of this cDNA yielded a novel protein of 551 amino acids (Fig. 2). The predicted protein contains a RING finger motif at the N-terminal, followed by two B-boxes, a coiled-coil domain and a distinct C-terminal region. This C-terminal domain is very proline-rich and has some similarity to the

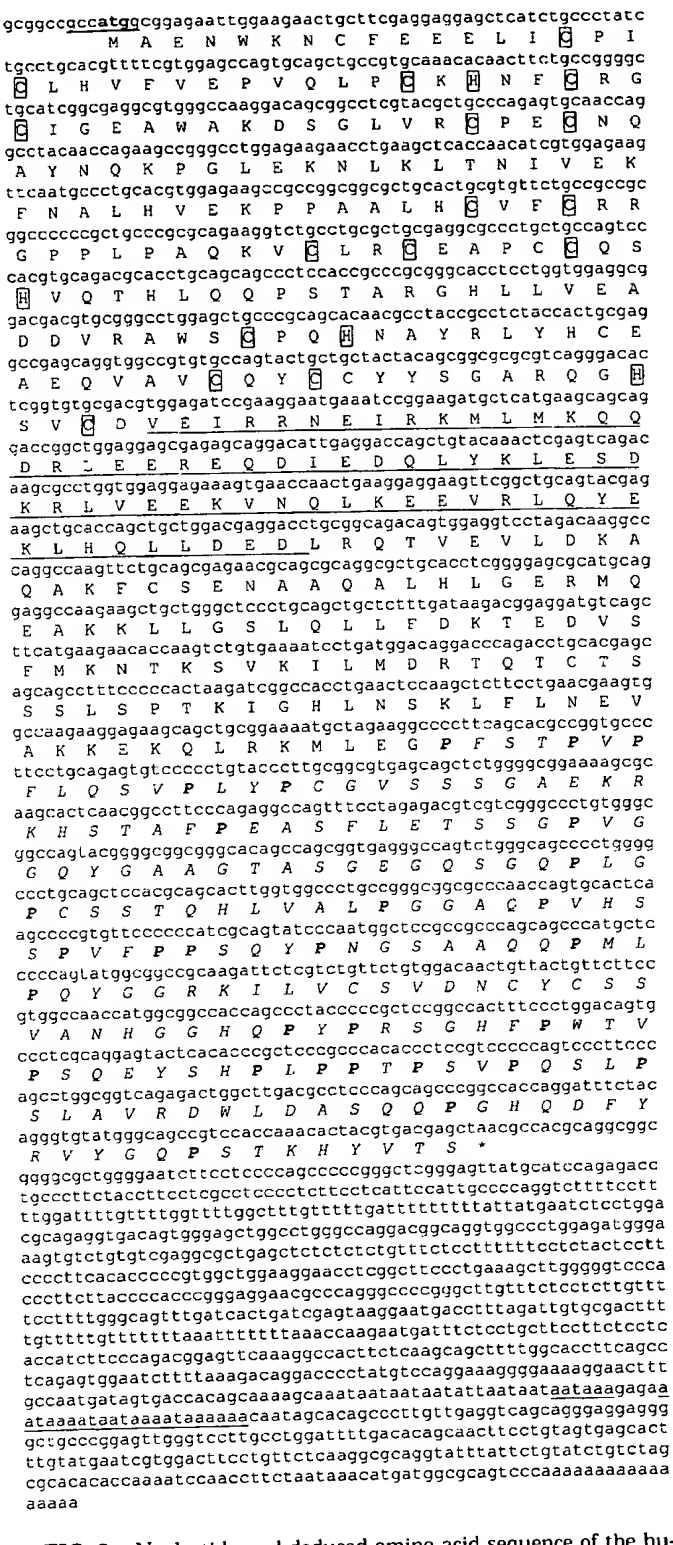

FIG. 2. Nucleotide and deduced amino acid sequence of the human GERP cDNA. The predicted initiation codon ATG, which is contained in a strong Kozak consensus sequence, is in bold, the conserved residues in the RING finger and B-boxes are boxed, the coiled-coil domain is underlined, and the proline-rich C-terminal region is in italics. Potential polyadenylation sites in the 3′-UTR are also underlined.

```
hgerp    1 MAENWKNCFEEELICPICLHVFVEPVQLPCKHNFCRGCIGEAWAKDSGLVRCPECNQAYN
mgerp    1 ............................................................

hgerp   61 QKPGLEKNLKLTNIVEKFNALHVEKPPAALHCVFCRRGPPLPAQKVCLRCEAPCCQSHVQ
mgerp   61 ........................T...................................

hgerp  121 THLQQPSTARGHLLVEADDVRAWSCPQHNAYRLYHCEAEQVAVCQYCCYYSGARQGHSVC
mgerp  121 ............................................................H......

hgerp  181 DVEIRRNEIRKMLMKQQDRLEEREQDIEDQLYKLESDKRLVEEKVNQLKEEVRLQYEKLH
mgerp  181 ................E.....................M.......S.............

hgerp  241 QLLDEDLRQTVEVLDKAQAKFCSENAAQALHLGERMQEAKKLLGSLQLLFDKTEDVSFMK
mgerp  241 ................G...........................R........G...

hgerp  301 NTKSVKILMDRTQTCTSSSLSPTKIGHLNSKLFLNEVAKKEKQLRKMLEGPFSTPVPFLQ
mgerp  301 ................G.....P.....................................

hgerp  361 SVPLYPCGVSSSGAEKRKHSTAFPEASFLETSSGPVGGQYGAAGTASGEGQSGQPLGPCS
mgerp  361 ........N.....................................S.............

hgerp  421 STQHLVALPGGAQPVHSSPVFPPSQYPNGSAAQQPMLPQYGGRKILVCSVDNCYCSSVAN
mgerp  421 ..........T................TT.............................

hgerp  481 HGGHQPYPRSGHFPWTVPSQEYSHPLPPTPSVPQSLPSLAVRDWLDASQQPGHQDFYRVY
mgerp  481 ....................................G.......................

hgerp  541 GQPSTKHYVTS
mgerp  541 ...........
```

FIG. 3. Alignment of the human (top) and mouse (bottom) GERP protein sequences. Only the differences between the two sequences are indicated.

B1

```
GERP     CVFCRRGPPLPAQKVCLRCEAPCCQSHVQT-HLQQPSTARGHLLVE
ATG-D    CDSCI-GNKQKAVKSCLVCQASFCELHLKP-HLEGAAF-RDHQLLE
TIF1α    CTSCEDNAEANGF--CVECVEWLCKTCIRA-HQRVKTFTKDHTVRQ
TIF1β    CTSCEDNAPATSY--CVECSEPLCETCVEA-HQRVK-YTKDHTVRS
TIF1γ    CTSCEDNASAVGF--CVECGEWLCKTCIEA-HQRVKFTK-DHLIRK
PML      CTRCKESA---DFW-CFECEQLLCAKCFEA-HQW----FLKHEARP
ARD1     CTVCATHL-------CSECSQVTHSTKTLAKHRRVPLADKPHEKTM
EFP      CDHCLKEAAVKT---CLVCMASFCQEHLQP-HFDSPA-FQDHPLQP
MID1     CQFCDQDPAQDAVKTCVTCEVSYCDECLKATHPNKKP-FTGHRLIE
MID2     CQFCEQDPPRDAVKTCITCEVSYCDRCLRATHPNKKP-FTSHRLVE
```

B2

```
GERP     CPQ--HNA--YRLYHCEAE----QVAVCQYCCYYSGA--HQGHSVC
ATG-D    CPV--HGK-TMELF-CQTD----QTCICYLCMFQE----HKNHSTV
TIF1α    CPF--HKKEQLKLY-CETC----DKLTCRDCQLLE----HKEHRYQ
TIF1β    CNV--HKHEPLVLF-CESC----DTLTCRDCQLNA----HKDHQYQ
TIF1γ    CPV--HKQEQLKLF-CETC----DRLTCRDCQLLE----HKEHRYQ
PML      CSNPNHRTPTLTSIYCRGC----SKPLCCSCALLDSSHSELKCDIS
ARD1     CSQ--HQVHAIEFV-CLEEGCQTSPLMCCVCKEYGK---HQGHKHS
EFP      CSQ--HNR-LREF-FCPEH----SECICHICLV-----EHKTCSPA
MID1     CLE--HEDEKV-NMYCVTD----DQLICALCKLVGR---HRDHQVA
MID2     CLD--HENEKV-NMYCVSD----DQLICALCKLVGR---HRDHQVA
```

FIG. 4. Alignment of the first (B1) and second (B2) B-box domains in the GERP protein, with those in the other RBCC proteins containing a pair of these motifs and with the ataxia-telangiectasia group D gene product (ATG-D). Conserved residues forming the B-box are in bold.

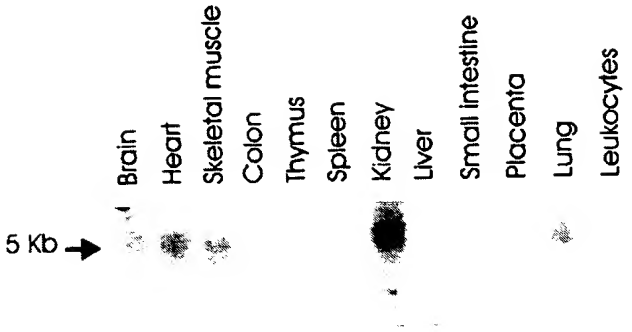

FIG. 5. Expression of GERP in various human tissues analyzed by Northern blotting. A premade blot containing about 1 μg of poly(A)$^+$ RNA per lane from various human tissues (Clontech) was used with a ^{32}P-labeled fragment of human GERP as a probe.

proline-rich repeats seen in the terminal of mucin (21), the homeobox protein HOX-B1 (22), Trypanosome neuraminidase (23), and the human putative tumor suppressor gene ZFM1 (24).

Reverse-transcriptase-PCR of mouse brain mRNA with the same primers also yielded a cDNA of 1.9 kb. Translation of the mouse cDNA sequence yielded a protein 97% identical to that from the human (Fig. 3). The calculated relative molecular mass (M_r) of the predicted human and mouse proteins are 61,508 and 61,591 Da, respectively. The sequences for the human and mouse cDNAs have been deposited in the GenBank/EBI Data Bank with Accession Nos. AF281046 and AF281047, respectively.

We have cloned a new, highly conserved member of the RING finger protein family. The sequence encodes an N-terminal RING finger, followed closely by two B-box motifs and a coiled-coil domain. Thus this protein belongs to the RBCC subclass of RING finger proteins (3). The two B-box domains show high homology to those in the other RBCC proteins containing two B-boxes (Fig. 4). The other proteins so far identified in this RBCC subfamily include PML (11–13), the TIF proteins (14, 15), the unusual GTPase, ARD1 (19), the estrogen-responsive finger protein, EFP (16) and MID1 (17) and MID2 (18). Mutations in MID1 are responsible for X-linked Opitz syndrome (17). Chromosomal translocations involving PML and TIF1α give rise to cell transformation (11–15). GERP also shows significant homology with the ataxia–telangiectasia group D gene product, which has two B-boxes followed by a coiled-coil, but lacks the N-terminal RING finger (24).

Our Northern blot analysis indicates that human GERP is expressed as a 5 kb transcript in kidney, as well as in brain, heart, skeletal muscle and lung. Lower levels of expression were also detected in placenta liver, leukocytes and intestine (Fig. 5). Ests corresponding to the GERP sequence have been described from mouse brain (AU67154), kidney (AI479267), mammary gland (AI021070), placenta (AA027381), myotubes (AI604031), embryos (AI115810) and embryonic ectoplacental cone (AA409281). Human sequences have been reported from germinal center B cells (AA482160), breast (H15126), placenta (R77167), fetal lung (AI336213), thymus (AI961791), and colonic mucosa (AI660262). Expression has also been noted in ovarian (AA411134), parathyroid (AA860536), lung (AA523517), and colon (AI917643) tumors, adenocarcinoma (AI925572), squamous cell carcinoma (AI830869), and anaplastic oligodendroglioma (AI828500, AI564811, AI871306, AI934897).

A sequence corresponding to the C-terminal two-thirds of the GERP sequence is also found in a contig that has been mapped to 10q24.3 (AC006106). Loss of heterozygosity is very often seen in the 10q24 region in human gliomas (25, 26). This suggests the presence of a tumor suppressor gene at this site. This is consistent with the finding that transfer of chromosome 10q into human glioblastoma cells suppresses the transformed and tumorigenic phenotype (27). The possible involvement of the GERP gene in 10q24.3 in this process remains to be determined.

ACKNOWLEDGMENT

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REFERENCES

1. Borden, K. L. B. (2000) *J. Mol. Biol.* **295,** 1103–1112.
2. Freemont, P. S. (2000) *Curr. Biol.* **10,** R84–R87.
3. Reddy, B. A., and Etkin, L. D. (1991) *Nucleic Acids Res.* **19,** 6330.
4. Takahashi, M., Inaguma, Y., Hiai, H., and Hirose, F. (1988) *Mol. Cell. Biol.* **8,** 1853–1856.
5. Ogawa, S., Goto, W., Orimo, A., Hosoi, T., Ouchi, Y., Muramatsu, M., and Inoue, S. (1998) *Biochem. Biophys. Res. Commun.* **251,** 515–519.
6. Chan, E. K. L., Hamel, J. C., Buyon, J. P., and Tan, E. M. (1991) *J. Clin. Invest.* **87,** 68–76.
7. Patarca, R., Freeman, G. J., Schwartz, J., Singh, R. P., Kong, Q. T., Murphy, E., Anderson, Y., Sheng, F. Y., Singh, P., Johnson, K. A., Guarnagia, S. M., Durfee, T., Blattner, F., and Cantor, H. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2733–2737.
8. Chu, T. W., Capossela, A., Coleman, R., Goei, V. L., Nallur, G., and Gruen, J. R. (1995) *Genomics* **29,** 229–239.
9. El-Husseini, A. E., and Vincent, S. R. (1999) *J. Biol. Chem.* **274,** 19771–19777.
10. Harada, H., Harada, Y., O'Brien, D. P., Rice, D. S., Naeve, C. W., and Downing, J. R. (1999) *Mol. Cell. Biol.* **19,** 3808–3815.
11. Kakizuka, A., Miller, W. H., Umesono, K., Warrell, R. P., Frankel, S. R., Murty, V. V., Dmitrovsky, E., and Evans R. M. (1991) *Cell* **66,** 663–674.
12. de The, H., Lavau, C., Marchio, A., Chomienne, C., Degos, L., and Dejean, A. (1991) *Cell* **66,** 675–684.
13. Goddard, A. D., Borrow, J., Freemont, P. S., and Solomon, E. (1991) *Science* **254,** 1371–1374.
14. Miki, T., Fleming, T. P., Crescenzi, M., Molloy, C. J., Blam, S. B., Reynolds, S. H., and Aaronson, S. A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5167–5171.

15. LeDouarin, B., Zechel, C., Garnier, J. M., Lutz, Y., Tora, L., Pierrat, P., Heery, D., Gronemeyer, H., Chambon, P., and Losson, R. (1995) *EMBO J.* **14**, 2020–2033.
16. Inoue, S., Orimo, A., Hosoi, T., Kondo, S., Toyoshima, H., Kondo, T., Ikegami, A., Ouchi, Y., Orimo, H., and Muramatsu, M. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 11117–11121.
17. Quaderi, N. A., Schweiger, S., Gaudenz, K., Franco, B., Rugarli, E. I., Berger, W., Feldman, G. J., Volta, M., Andolfi, G., Gilgenkrantz, S., Marion, R. W., Hennekam, R. C., Opitz, J. M., Muenke, M., Ropers, H. H., and Ballabio, A. (1997) *Nat. Genet.* **17**, 285–291.
18. Buchner, G., Montini, E., Andolfi, G., Quaderi, N., Cainarca, S., Messali, S., Bassi, M. T., Ballabio, A., Meroni, G., and Franco, B. (1999) *Hum. Mol. Genet.* **8**, 1397–1407.
19. Mishima, K., Tsuchiya, M., Nightingale, M. S., Moss, J., and Vaughan, M. (1993) *J. Biol. Chem.* **268**, 8801–8807.
20. Kozak, M. (1986) *Cell* **44**, 283–292.
21. Park, H., Hyun, S. W., and Kim, K. C. (1996) *Am. J. Respir. Cell Mol. Biol.* **15**, 237–244.
22. Acampora, D., D'Esposito, M., Faiella, A., Pannese, M., Migliaccio, E., Morelli, F., Stornaiuolo, A., Nigro, V., Simeone, A., and Boncinelli, E. (1989) *Nucleic Acids Res.* **17**, 10385–10402.
23. Wrehlke, C., Schmitt-Wrede, H. P., Qiao, Z., and Wunderlich, F. (1997) *DNA Cell Biol.* **16**, 761–767.
24. Leonhardt, E. A., Kapp, L. N., Young, B. R., and Murnane, J. P. (1994) *Genomics* **19**, 130–136.
25. Rasheed, B. K., Fuller, G. N., Friedman, A. H., Bigner, D. D., and Bigner, S. H. (1992) *Genes Chromosomes Cancer* **5**, 75–82.
26. Chernova, O., and Cowell, J. K. (1998) *Cancer Genet. Cytogenet.* **105**, 60–68.
27. Steck, P. A., Lin, H., Langford, L. A., Jasser, S. A., Koul, D., Yung, W. K., and Pershouse, M. A. (1999) *Genes Chromosomes Cancer* **24**, 135–143.

Exhibit C

Atlas of **Breast Cancer** Second Edition

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CONTENTS

9

Pathology of breast cancer

Stuart J. Schnitt

More than 95 percent of breast malignancies arise from the breast epithelial elements and are classified as carcinomas (or specifically, adenocarcinomas). Though often discussed as if it were a single disease, "breast carcinoma" actually encompasses a diverse group of lesions that differ in microscopic appearance and biologic behavior. Breast carcinomas can be divided into two major groups: noninvasive (in situ) cancers, in which the tumor cells remain confined to the ducts or lobules and show no light microscopic evidence of invasion into the surrounding stroma, and invasive (infiltrating) cancers, characterized by tumor cells that invade the breast stroma.

The noninvasive lesions are of two types: ductal carcinoma in situ (also known as intraductal carcinoma), and lobular carcinoma in situ. These two types of lesions

Figure 9.1 Comparative features of ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS)

	DCIS	LCIS
Presentation	Incidental finding, mammographic abnormality, nipple discharge, Paget's disease, palpable mass	Incidental finding
Predominant location	Ducts	Lobules
Cell size	Medium or large	Small
Pattern	Comedo, cribriform, micropapillary, papillary, solid	Solid
Calcifications	Present or absent	Usually absent
Risk of subsequent invasive cancer	Higher	Lower
Location of subsequent invasive cancer	Ipsilateral	Ipsilateral or contralateral

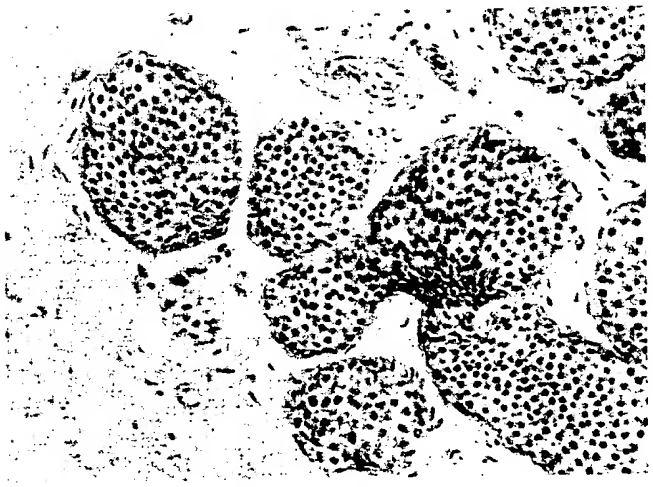


Figure 9.2 LCIS. The acini of this lobule are filled with and distended by a population of small, uniform, round tumor cells with bland nuclei. LCIS is typically multicentric and bilateral. Patients with this lesion have about a 1 percent per year risk of developing invasive cancer.

differ in their clinical features, morphology, and biologic behavior (Fig. 9.1). Whereas the microscopic appearance of lobular carcinoma in situ is stereotypical (Fig. 9.2), ductal carcinoma in situ is histologically heterogeneous with regard to architectural pattern and nuclear grade (Figs. 9.3 and 9.4). These morphologic differences are also

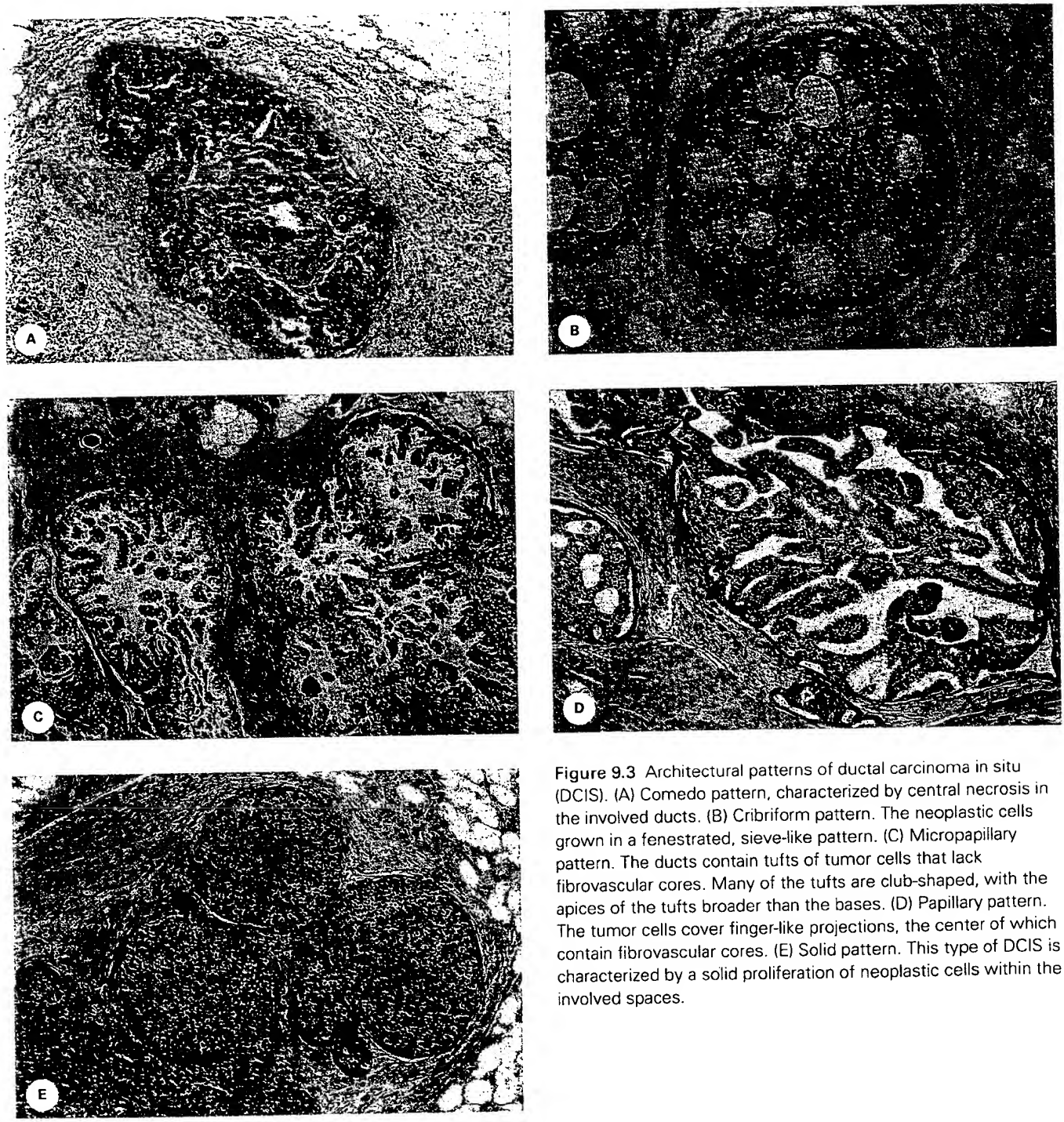


Figure 9.3 Architectural patterns of ductal carcinoma in situ (DCIS). (A) Comedo pattern, characterized by central necrosis in the involved ducts. (B) Cribriform pattern. The neoplastic cells grown in a fenestrated, sieve-like pattern. (C) Micropapillary pattern. The ducts contain tufts of tumor cells that lack fibrovascular cores. Many of the tufts are club-shaped, with the apices of the tufts broader than the bases. (D) Papillary pattern. The tumor cells cover finger-like projections, the center of which contain fibrovascular cores. (E) Solid pattern. This type of DCIS is characterized by a solid proliferation of neoplastic cells within the involved spaces.

reflected in the profile of biological markers exhibited by ductal carcinoma in situ lesions (Fig. 9.5).

The invasive breast carcinomas also comprise a heterogeneous group of lesions. The most common type is

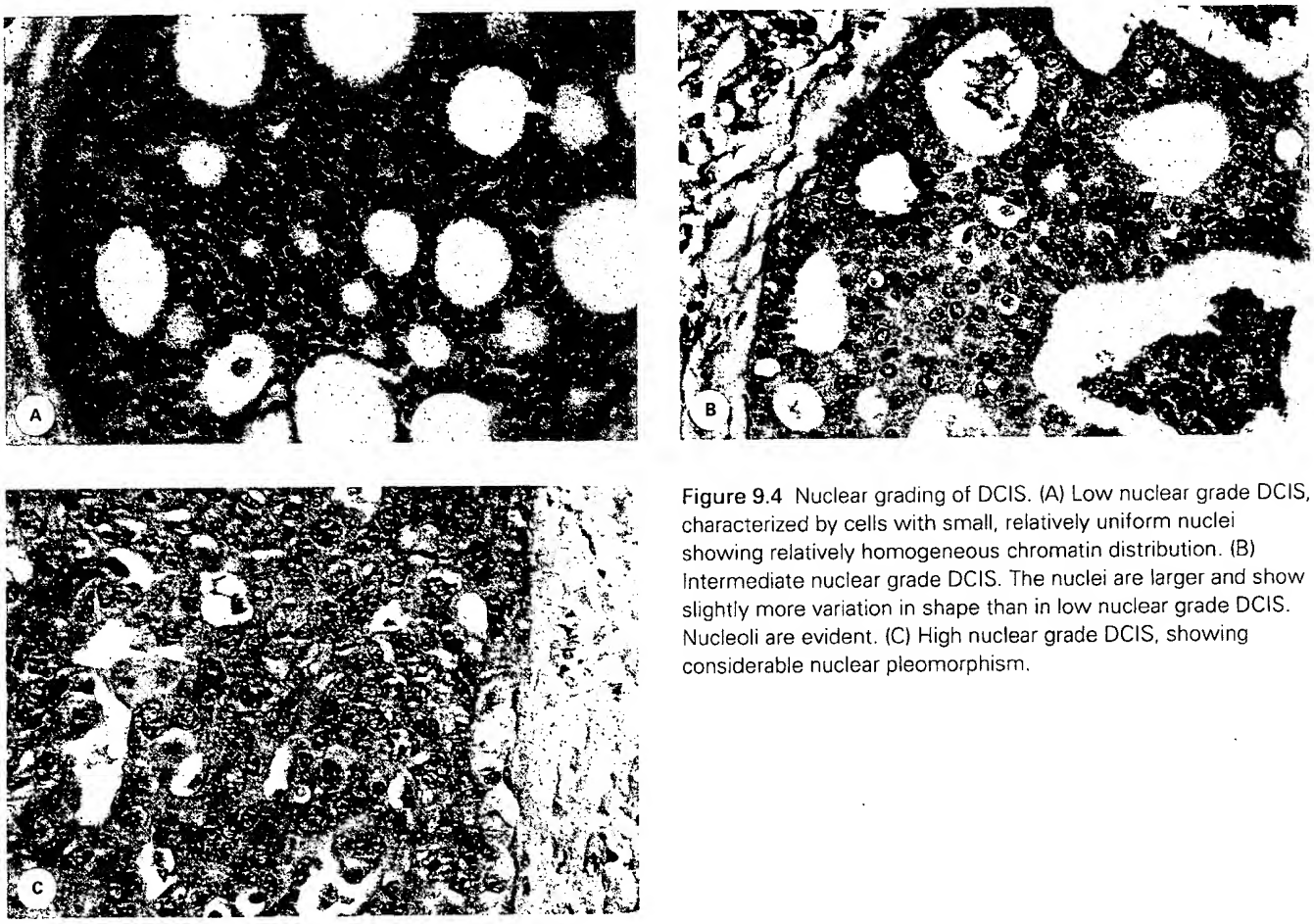


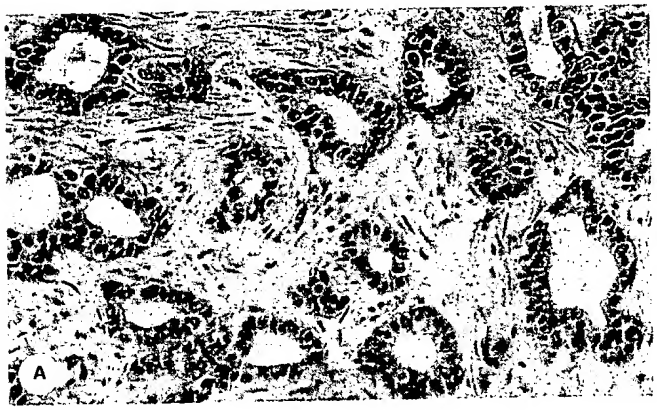
Figure 9.4 Nuclear grading of DCIS. (A) Low nuclear grade DCIS, characterized by cells with small, relatively uniform nuclei showing relatively homogeneous chromatin distribution. (B) Intermediate nuclear grade DCIS. The nuclei are larger and show slightly more variation in shape than in low nuclear grade DCIS. Nucleoli are evident. (C) High nuclear grade DCIS, showing considerable nuclear pleomorphism.

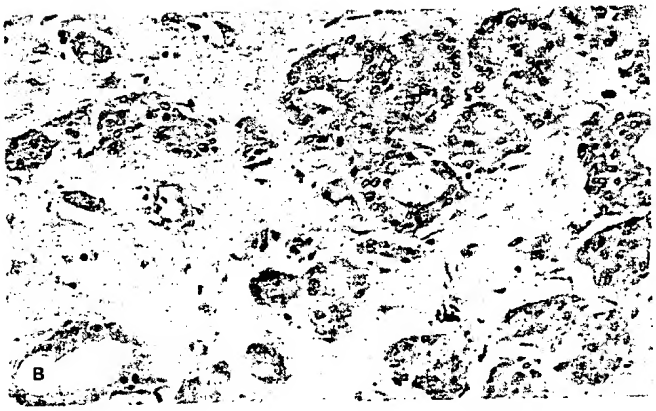
Figure 9.5 Biological markers in ductal carcinoma in situ

	High grade	Low grade
Aneuploidy	Frequent	Infrequent
Proliferative rate	High	Low
Hormone receptors	Positive	Negative
HER-2/*neu* oncogene expression	Frequent	Infrequent
P53 gene abnormalities	Frequent	Infrequent
Angiogenesis	Frequent	Infrequent

infiltrating ductal carcinoma (also termed "infiltrating carcinoma of no special type" or "infiltrating carcinoma", not otherwise specified). These tumors are usually hard, gray, gritty masses that invade the surrounding tissue (Fig. 9.6). On microscopic examination, infiltrating ductal carcinomas are divided into three grades—well-differentiated (grade I), moderately differentiated (grade II), and poorly differentiated (grade III)—based on a combination of architectural and cytologic features (Fig. 9.7). The prognostic significance of this grading system has been repeatedly documented in clinical follow-up studies. Approximately 20 percent of invasive breast cancers are considered "special types." Infiltrating lobular carcinomas are the second most common type of invasive breast

Figure 9.6 Macroscopic appearance of an infiltrating ductal carcinoma in a mastectomy specimen. The lesion is firm and shows extensions into the adjacent fat. This macroscopic appearance has been termed "scirrhous." (From Hayes, 1991.)





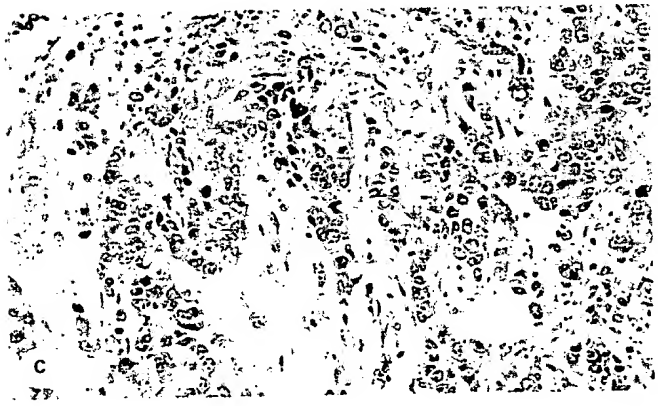


Figure 9.7 Infiltrating ductal carcinoma. (A) Well-differentiated (grade I). The tumor cells grow in glandular configurations. (B) Moderately differentiated (grade II). There is a mixture of well-formed glands and more solid tumor cell nests. (C) Poorly differentiated (grade III). The tumor is composed entirely of solid nests of tumor cells without evidence of gland formation.

cancer (Fig. 9.8). These tumors have a higher frequency of bilaterality and multicentricity than infiltrating ductal carcinomas. Although some authors have reported that the prognosis for infiltrating lobular cancers is similar to that of invasive ductal lesions, others believe that the classical cases of infiltrating lobular carcinomas with low-grade nuclei are a prognostically favorable subset. Other variants of invasive breast carcinoma, such as mucinous

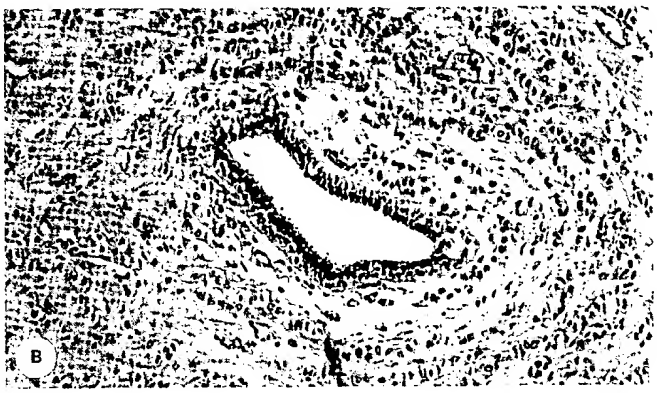


Figure 9.8 Infiltrating lobular carcinoma (classical type). (A) This tumor is characterized by small tumor cells that infiltrate the stroma in a single-file pattern. (B) The tumor cells in this section grow in a target-like pattern around a normal breast duct. (From Hayes, 1991.)

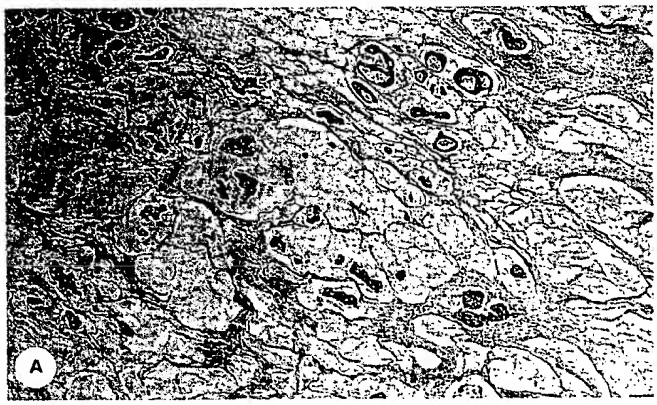


Figure 9.9 Mucinous (colloid) carcinoma. (A) Low-power view demonstrates islands of tumor cells within a sea of mucin. (B) Higher magnification shows tumor cell nests. The cells comprising this type of tumor are typically uniform in appearance. (From Hayes, 1991.)

Figure 9.10 Tubular carcinoma. This type of breast cancer is composed of tubular structures infiltrating the stroma. The tubules tend to be elongated, and many have pointed ends. The cells composing the tubules are cuboidal to columnar and often have apical cytoplasmic protrusions or "snouts". (From Hayes, 1991.)

(colloid), tubular, and papillary carcinomas, all have a better prognosis than infiltrating ductal carcinomas (Figs. 9.9 and 9.10). Medullary carcinomas are prognostically more favorable than poorly differentiated (grade III) infiltrating ductal carcinomas (Fig. 9.11). More unusual variants of invasive breast cancer include metaplastic carcinomas (Fig. 9.12) and adenoid cystic carcinomas (Fig. 9.13). The frequency of the more common types of invasive breast carcinoma in

Figure 9.11 Medullary carcinoma. This type of breast carcinoma is characterized by macroscopic and microscopic circumscription, poorly differentiated (high-grade) tumor cells that grow in a syncytial pattern, and a diffuse lymphoplasmacytic infiltrate. (A) Low-power photomicrograph demonstrates circumscribed tumor border. (B) High-power view demonstrates large, pleomorphic tumor cells and associated lymphocytes and plasma cells.

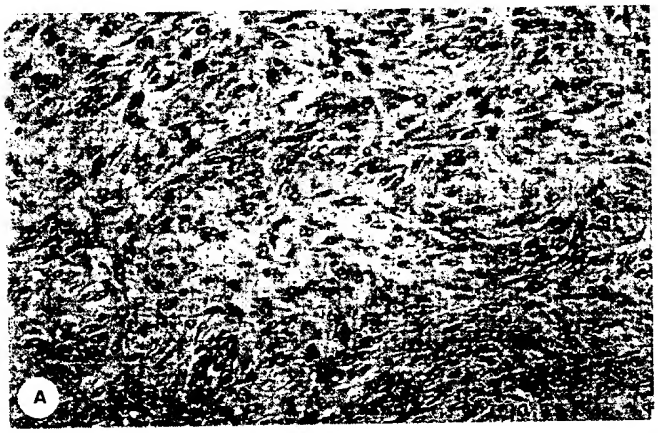


Figure 9.12 Metaplastic carcinomas. These are unusual variants of invasive breast cancers in which the tumor cells are of a type other than the glandular cells that comprise the common types of breast cancer. Tumor cells may be squamous, spindle-shaped, or may form cartilage or bone. (A) Metaplastic carcinoma composed primarily of spindle cells. (B) Metaplastic carcinoma with differentiation toward cartilage.

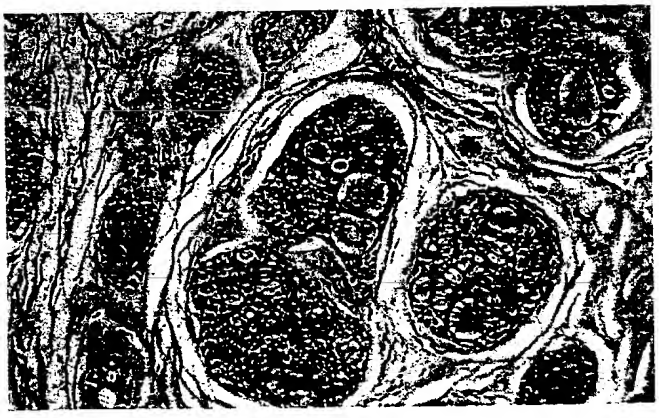


Figure 9.13 Adenoid cystic carcinoma. This type of invasive cancer is characterized by tumor cell nests containing glandular spaces. Within many of the gland lumina are hyaline deposits. These tumors have a better prognosis than the usual infiltrating ductal carcinomas.

two large studies is shown in Figure 9.14. It should be noted, however, that these series represent breast cancers diagnosed in the premammographic era. Certain histologic types, particularly tubular carcinoma, are relatively more frequent in mammographically-screened populations.

A number of histologic features of breast carcinomas have been reported to be associated with an unfavorable prognosis. These include histologic grade, lymphatic vessel and blood vessel, among others (Fig. 9.15). An extensive intraductal component within an infiltrating ductal carcinoma has been associated with an increased risk of tumor recurrence in the breast after conservative surgery and radiation therapy (see Chapter 10).

A few types of breast carcinoma merit special attention. Paget's disease is a disorder of the nipple and areola, characterized clinically by an eczematoid appearance

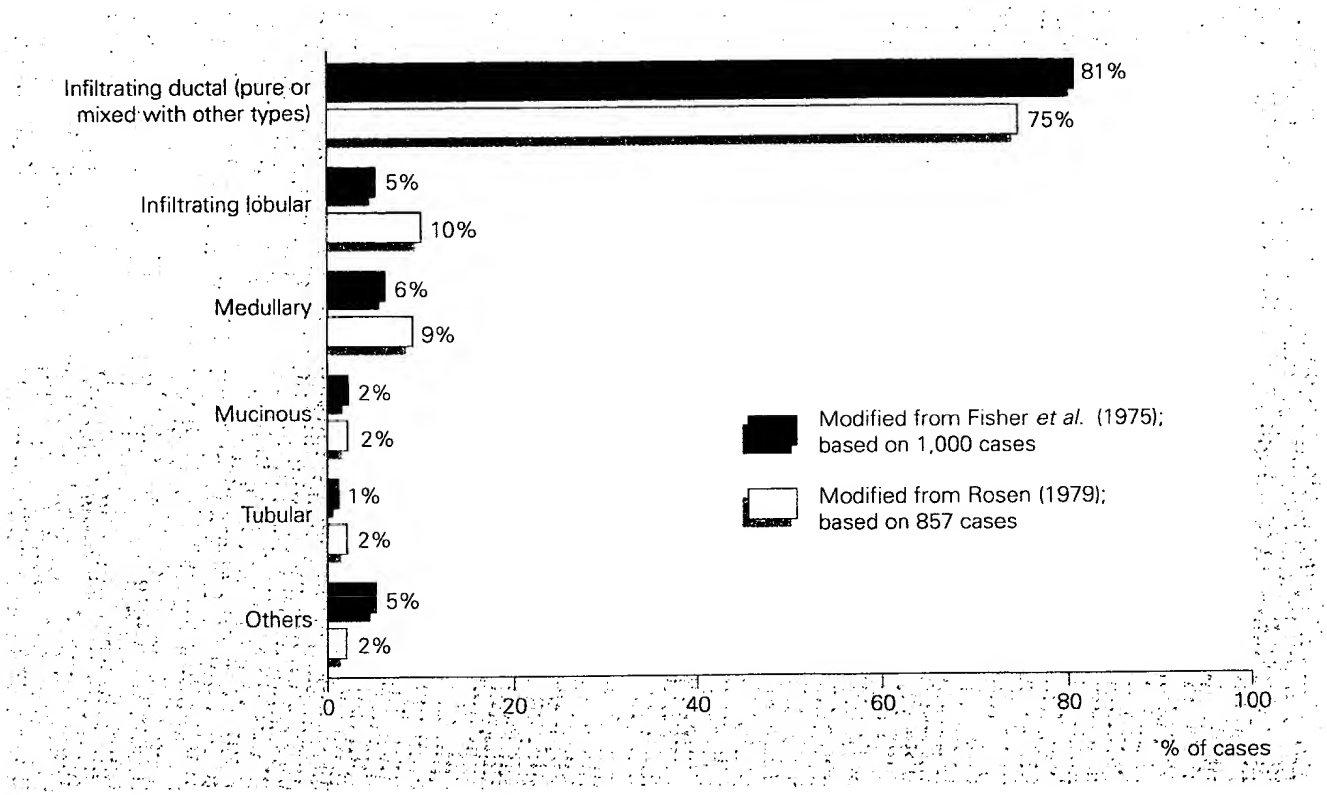


Figure 9.14 Frequency of histologic types of invasive breast cancer in two large series.

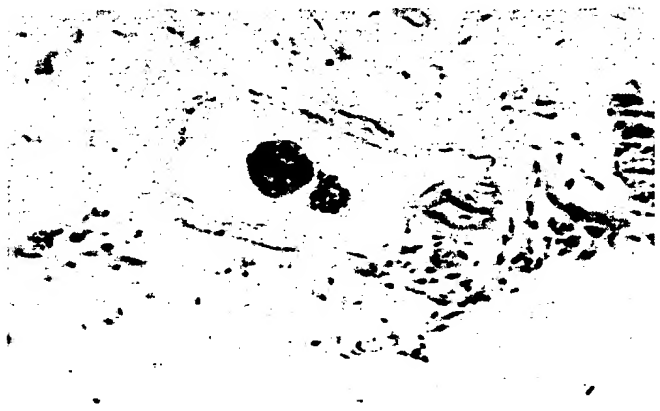
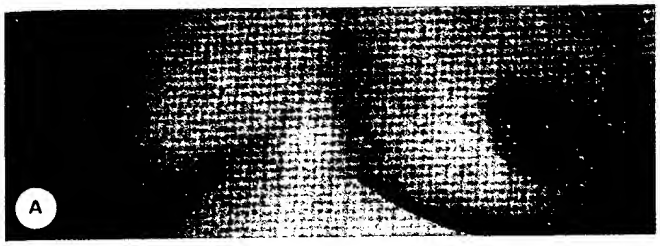


Figure 9.15 Lymphatic vessel invasion adversely affects the prognosis in patients with invasive breast cancer.

with crusting, scaling, or erosion. Histologically, breast cancer cells are found within the nipple and the areolar epidermis (Fig. 9.16). This lesion is almost always associated with an underlying mammary carcinoma, which may be either in situ or invasive. Inflammatory carcinoma is a form of locally advanced breast cancer characterized clinically by erythema, edema, and warmth of the skin of the breast. Histopathologic examination of the skin typically demonstrates dermal lymphatic invasion by tumor cells (Fig. 9.17).



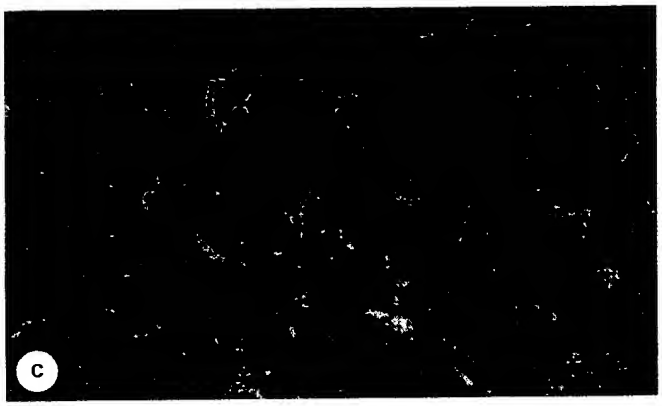


Figure 9.16 Paget's disease. Clinically, patients with this disorder present with an eczematous lesion of the nipple that may also involve the areola. This lesion is typically associated with an underlying breast cancer. (A) Photograph demonstrates eczematoid change of the left nipple and areola. (From Hayes, 1991.) (B) The epidermis is infiltrated by cancer cells with abundant pink cytoplasm and large, vesicular nuclei with prominent nucleoli. (C) High-power view demonstrates tumor cells within the epidermis.

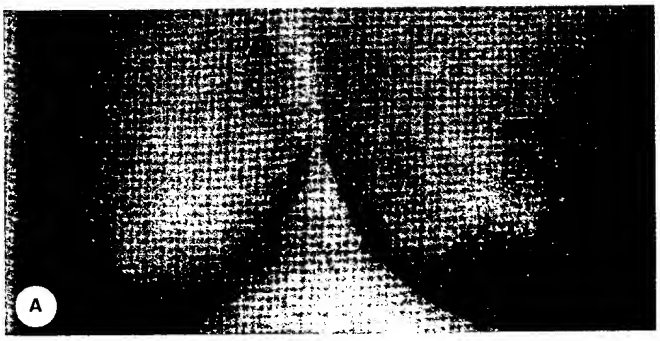
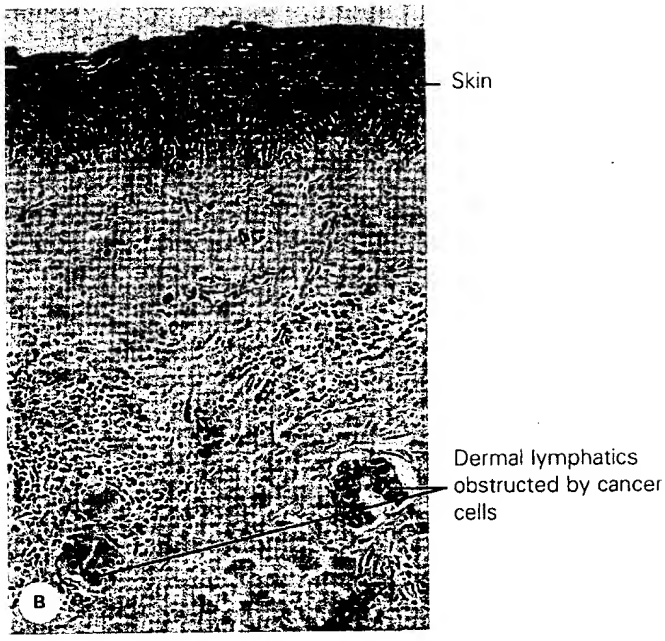
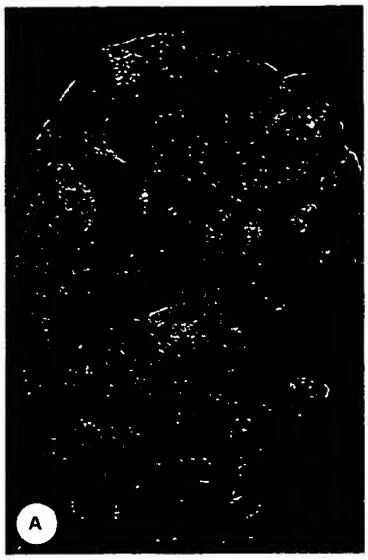


Figure 9.17 Inflammatory carcinoma. (A) Classically, inflammatory breast cancer does not present as a discrete mass, but rather as cutaneous erythema with overlying skin warmth, as illustrated in the left breast of this 63-year-old patient. (B) Pathologic confirmation of invasion of dermal lymphatics by malignant cells, as shown in this photomicrograph, can help distinguish this condition from benign mastitis. The erythema and warmth observed clinically are due to obstruction of dermal lymphatics and subsequent cutaneous lymphedema. (From Hayes, 1991.)



OTHER BREAST NEOPLASMS

Phyllodes tumors (cystosarcoma phyllodes) are tumors composed of a mixture of hypercellular stroma and benign ductal structures. The stroma is the neoplastic element in these tumors, and it may be cytologically bland or frankly sarcomatous (Fig. 9.18). Angiosarcomas are uncommon malignant vascular tumors. These lesions, particularly when of high grade, are extremely aggressive (Fig. 9.19). Other types of sarcomas may arise in the breast but are extremely rare. Lymphomas may involve the breast either as primary tumors or in conjunction with lymphoma in extramammary sites (Fig. 9.20).



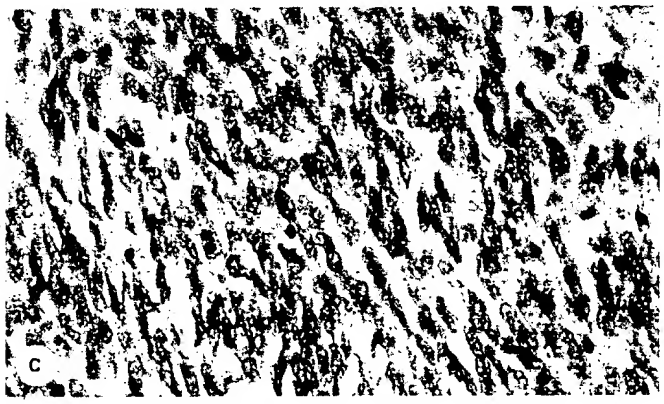


Figure 9.18 Phyllodes tumor (cystosarcoma phyllodes). (A) These lesions are circumscribed masses which, on cut surface, show cleft-like spaces intermingled with tan–yellow nodules of tumor. (From Hayes, 1991.) (B) Low-power microscopic examination reveals that the tumor is composed primarily of hypercellular stroma. A benign ductal structure is also seen. (C) High-power view shows atypical stromal cells with one mitotic figure. The stroma may be benign or malignant. When malignant, the stroma may be composed of fibrosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, or rhabdomyosarcoma.

Figure 9.19 Angiosarcoma. (A) In this relatively well-differentiated angiosarcoma, interanastomosing vascular spaces infiltrate the breast stroma. (B) Higher-power view shows papillary projections of malignant endothelial cells within the vascular spaces.

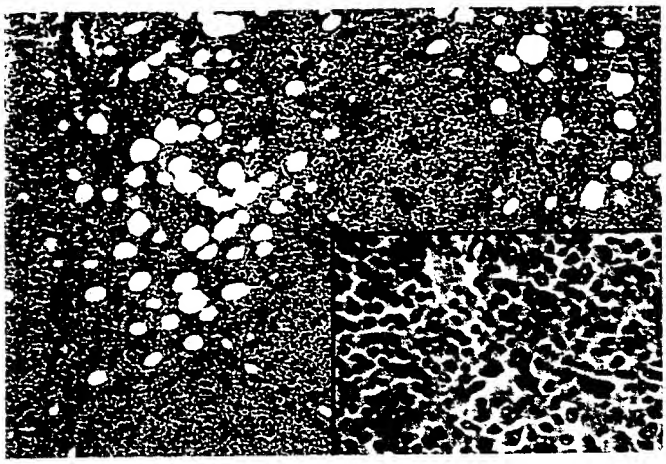


Figure 9.20 Lymphoma. The breast tissue is infiltrated by atypical lymphoid cells in a nodular and diffuse pattern. The inset shows an example of a follicular center cell lymphoma, mixed small-cell and large-cell type.

SUGGESTED READING

Fisher ER, Gregorio RM, Fisher B, et al. (1975) The pathology of invasive breast cancer. A syllabus derived from findings from the National Surgical Adjuvant Breast Project (Protocol No. 4). *Cancer* 36:1–85.

Hayes DF. (1991) Breast cancer. In: Skarin AT, ed. *Atlas of Diagnostic Oncology*. New York: Gower Medical Publishing, 6.1–6.31.

Page DL. (1991) Prognosis and breast cancer. Recognition of lethal and favorable prognostic types. *Am J Surg Pathol* 15:334–349.

Rosen PP. (1979) The pathological classification of human mammary carcinoma: past, present and future. *Ann Clin Lab Sci* 9:144–156.

Rosen PP. (1991) The pathology of invasive breast carcinoma. In: Harris JR, Hellman S, Henderson IC, Kinne DW, eds. *Breast Diseases,* 2nd ed. Philadelphia: JB Lippincott, 245–296.

Schnitt SJ. Pathology of in situ carcinoma. In: Harris JR, Lippman ME, Morrow M, Osborne K, eds. *Diseases of the Breast,* 2nd ed. Philadelphia: Lippincott-Raven, in press.

Schnitt SJ, Guidi AJ. Pathology of invasive breast cancer. In: Harris JR, Lippman ME, Morrow M, Osborne K, eds. *Diseases of the Breast,* 2nd ed. Philadelphia: Lippincott-Raven, in press.

Schnitt SJ. (1991) Pathology of in situ carcinoma. In: Harris JR, Hellman S, Henderson IC, Kinne DW, eds. *Breast Diseases,* 2nd ed. Philadelphia: JB Lippincott, 229–232.

Silverstein ML, ed. (1997) *Ductal Carcinoma In Situ of the Breast.* Baltimore: Williams & Wilkins.